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Stimulation of Body Weight Increase and Epiphyseal Cartilage Growth by Insulin-Like Growth Factor

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observer-output error vector, with $\| \cdot \|$ or $- \cdot -$ to it. In order to cope with these cases, a secondary observer is synthesized to reconstruct the entire observer-error vector from the observer output error vector. This approach leads toward the design of highly sensitive and reliable FDS, with the possibility of obtaining a unique fingerprint for every possible failure.

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STIMULATION OF BODY WEIGHT INCREASE AND EPIPHYSEAL
CARTILAGE GROWTH BY INSULIN-LIKE GROWTH FACTOR

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SUMMARY

The ability of insulin-like growth factor (IGF) to induce growth in hypophysectomized immature rats was tested by continuous infusion of the partially purified factor at daily doses of 6, 21, and 46 mU for an 8-day period. A dose-dependent growth of the proximal epiphyseal cartilage of the tibia and an associated stimulation of the primary spongiosa were produced by these amounts of IGF. The two highest doses of IGF also resulted in dose-dependent increases of body weight. Gel permeation of the sera at neutrality showed that the large-molecular-weight IGF binding protein was not induced by the infusion of IGF, whereas it was generated in the sera of hypophysectomized rats that had been infused with daily doses of 86 mU of human growth hormone.

INTRODUCTION

According to the somatomedin hypothesis (ref. 1), longitudinal body growth is stimulated by the direct action of somatomedin on epiphyseal chondrocytes. The plasma concentrations of somatomedin or insulin-like growth factors and their binding proteins are regulated by pituitary growth hormone (refs. 2 and 3). Although there is a considerable body of evidence from in vitro studies that supports the somatomedin hypothesis (refs. 4 and 5), the demonstration of growth stimulation in the whole animal by somatomedin peptides has been lacking until recently. Using Snell dwarf mice, van Buul-Offers and Van den Brande (ref. 6) showed that longitudinal skeletal growth and body weight gain could be stimulated by the injection of a somatomedin preparation for 4 weeks. It is reported herewith that immature hypophysectomized rats, infused with insulin-like growth factor (IGF), show body weight gains and epiphyseal cartilage proliferations similar to that produced by pituitary growth hormone.

MATERIALS AND METHODS

Male albino rats weighing between 85 and 95 g were hypophysectomized and checked for weight gain during the ensuing 2- to 3-week period. Rats that gained not more than 0.5 g/day were considered completely hypophysectomized and were selected for assay. Testicular weights at autopsy ranged between 150 and 200 mg and served to confirm the completeness of hypophysectomy. The rats had free access to commercial rat chow (Altromin, Lage, Fed. Rep. Germany) and water and were maintained on a 12-hr light and dark cycle.

The partially purified preparation of IGF used in these studies has a specific activity of 13 mU of insulin equivalents per milligram, as standardized with the fat pad assay of Froesch et al. (ref. 7). It had been processed through the Sephadex gel

filtration steps in the purification scheme devised by Rinderknecht and Humbel (ref. 8). The content of the immunoreactive IGF I and II in the 13 mU/mg preparation as determined in this laboratory was 73% and 27%, respectively.

Clinical grade human growth hormone (hGH) was purchased from Leo Pharmaceuticals (Denmark). The preparation was dissolved in sterile saline, loaded into Alzet mini-pumps of 240 μ l capacity (No. 2001) (Alza, Palo Alto, California) and, after 2 to 3 hr soaking in sterile saline, were implanted subcutaneously into hypophysectomized rats under light ether anaesthesia. The minipumps were rated to deliver 1 μ l/hr. The IGF preparation was administered at three different concentrations, namely, 46, 21, and 6 mU/day from minipumps containing, respectively, 35, 16.3, and 4.8 mg of the IGF preparation per pump. Human GH was infused at the rate of 86 mU/day. The rats were sacrificed on the ninth day after implantation and the pumps dismantled to confirm that the contents had been delivered. The tibia was removed and stained according to the method of Evans et al. (ref. 9). In addition, the testes, thymus, liver, soleus muscle, and the retroperitoneal and epididymal fat pads were dissected and weighed. The significances of means were evaluated by the Student's t-test.

Blood samples were drawn at autopsy from the abdominal aorta of the rats under ether anaesthesia and the serum was used for the determination of the IGF binding protein pattern according to the radiochromatographic method of Kaufmann et al. (ref. 2) modified for in vitro use. Whereas these authors injected the IGF tracer intravenously into the rats and chromatographed the serum 20 min after the injection, in the present study the tracer was added directly to the rat serum, equilibrated for 24 hr at 4°C, and then applied to Sephadex G-200 columns at neutral pH as given in the legend to figure 4. This procedure yields a binding pattern that, quantitatively, is slightly different from that obtained after the in vivo administration of the IGF tracer with respect to the relative heights of the radioactive peaks II and III — peak III being higher than peak II for normal serum and for serum from the hypophysectomized rats treated with GH. However, qualitatively, the binding patterns obtained from the present study are similar to those reported earlier by Kaufmann et al. (ref. 2) and more recently, by Moses et al. (ref. 3).

RESULTS

The body weight changes associated with the infusion of 21 and 46 mU of IGF per day for 8 days are shown in figure 1. A dose of 6 mU of IGF produced no increase in body weight, although small but significant increases in epiphyseal cartilage width were consistently detectable, as noted below. A noteworthy feature of the IGF effects is that no weight gain occurred during the first 2 days of infusion. This contrasts with the immediate weight gain effected by the hGH infusion. The reason for this difference is not apparent at this time. It appears from these curves that the rate of weight gain induced by the infusion of IGF at the rate of 46 mU nearly equals that given by 86 mU of hGH, but the maximal response was smaller.

From the standpoint of epiphyseal response, the infusion of 6 mU of IGF per day appears to be the minimal effective dose for eliciting a significant response (20%, $P < 0.05$ vs control). The lack of a body weight gain at this dose is not unexpected in view of the lower sensitivity of body weight responses compared to that of the epiphyseal cartilage. When the dose of infused IGF was increased to 21 mU/day, the epiphyseal cartilage response increased to 39% of control, corresponding to a body weight increase of 4 g; when the dose was increased to 46 mU/day, the cartilage response increased to 50% of control, corresponding to a body weight increase of 12 g. A log

dose-response relationship for the epiphyseal cartilage response is shown in figure 2. The marked proliferation of the epiphyseal cartilage plate after treatment with the high dose of IGF is shown in the silver-stained section of the tibias from treated and control rats (fig. 3). It is evident that the zone of provisional calcification and the primary spongiosa were stimulated by IGF in a manner very similar to that produced by growth hormone.

The highest dose of IGF also produced a significant increase in the wet weights of the liver, thymus, and soleus muscle (table 1). In this respect the IGF resembled the response given by growth hormone. If the weight of these organs was expressed as a percentage of body weight, then the values were not significantly different, indicating that the organ weights increased in proportion to the body weight. IGF decreased the absolute weights of the epididymal fat pads although the change at the highest dose was not significant due to the small number of animals. When calculated relative to 100 g body weight, the epididymal fat pads lost 50% of their weight ($p < 0.01$) at the highest dose of IGF. These reductions in epididymal fat pad weight are unexpected in the light of the antilipolytic action of IGF and requires investigation with pure IGF.

Since sera from hypophysectomized rats have been found to be deficient in high molecular weight binding protein that can be restored by treatment with growth hormone (refs. 2 and 3), it was considered of interest to determine whether IGF infusion could also repair the deficiency. For this purpose, pooled sera injected with the high dose of IGF was submitted to radiochromatographic analysis on G-200 following the *in vitro* incubation of the sera with ^{125}I -labeled IGF for 24 hr at 4°C. In figure 4 is shown a comparison of the elution profiles of the labeled peaks from the sera of normal rats and of hypophysectomized rats treated with IGF or hGH, or with human serum albumin as control. Normal serum displayed five peaks of radioactivity in the elution profile: I, nonspecifically bound IGF; II and III, the labeled IGF that bound specifically to a large molecular weight region ($\approx 200,000$) and to a lower molecular weight region, respectively; peak IV represented free labeled IGF, whereas peak V at 100% of the bed volume contained free iodine and iodinated degradation products of IGF. As shown in figure 4, in the presence of an excess of cold IGF (1 mU) the binding to peaks II and III was practically abolished, whereas the activity in peak IV was drastically increased by the displacement of labeled IGF from the peaks with specific binding.

In contrast to the serum pattern from normal rats, the pattern from untreated hypophysectomized rats revealed only four peaks of radioactivity, peak II being absent. Infusion of hGH (86 mU/day for 8 days) restored the binding in peak II. However, infusion of the high dose of IGF (46 mU/day for 8 days) did not repair the deficiency in peak II (fig. 4) despite a significant body weight gain and epiphyseal response.

DISCUSSION

The present data demonstrate that hypophysectomized rats can grow in response to a continuously administered preparation of a crude IGF prepared from human plasma. In view of the low purity of this preparation (13 mU/mg as compared to 330 mU/mg for pure IGF I) as well as the presence of both types of IGF, these experiments need to be repeated with pure IGF I and II. With regard to the possibility that contaminants such as insulin or thyroxin may have elicited the observed responses, it should be noted that these hormones do not stimulate dose-dependent growth in the

hypophysectomized rat. Although in the Snell dwarf mouse thyroxin does stimulate body weight gain and increase in skeletal length, in hypophysectomized rats its effect on growth is to synergize the action of pituitary growth hormone (ref. 10). The effects of pure IGF I were reported by Schoenle et al. (ref. 11) while the present manuscript was in revision. Their results showed that the infusion of the highly purified peptide stimulated epiphyseal cartilage growth, body weight gain, and H^3 -thymidine incorporation into rib cartilage of hypophysectomized rats. These results taken together indicate that the growth promoting effects obtained with crude IGF can be duplicated with essentially the equivalent unit age of pure IGF I, that is, the infusion of IGF on the order of 15 to 50 mU (fat pad assay) per day per 100 g body weight.

Since the present preparation is a mixture of IGF I and II, administration of the pure IGF types individually will be required to ascertain whether both hormones given alone elicit the same qualitative as well as quantitative growth responses *in vivo*. In this regard it may be noted that in several *in vitro* systems (chick embryo fibroblasts, chick cartilage, human embryonic lung fibroblasts, and human adult fibroblasts) the response to the two growth factors is similar (refs. 12 through 14), whereas in others (rat cartilage and calvarian cells) (Schmid and Froesch, *in preparation*) IGF I is more potent than IGF II. Also, disorders of growth hormone secretion are reflected more markedly by changes of the plasma concentrations of IGF I than of IGF II (ref. 15).

A growth response of Snell dwarf mice to a partially purified preparation of somatomedin, quantified by radioimmunoassay for the content of other hormonally active contaminants, has been reported by van Buul-Offers and Van den Brande (ref. 6). They injected 6 units of activity (porcine cartilage assay) for 4 weeks and obtained a significant increase in body length and weight comparable to that obtained with 8.3 ug of hGH or with 0.1 ug/day of thyroxin. A dose dependency of growth rate on somatomedin dose was not tested. Holder et al. (ref. 16) also reported a significant body weight gain after treating dwarf mice with somatomedin for 10 days; however, bone growth as measured by tail, femur, and tibia lengths was not significantly increased, perhaps because of the shorter time of injection as compared to that of van Buul-Offers.

It is of interest to compare the doses of IGF used in the hypophysectomized rats with those reported for Snell dwarf mice. If the doses of somatomedin administered are calculated per 100 g of body weight by means of the relationship that 1 costal cartilage somatomedin unit in 1 ml of pooled human plasma is equivalent to 0.2 mU of IGF by the fat pad assay (refs. 17 and 18), then the doses of activity expressed as IGF per day per 100 g body weight are the same order of magnitude in the different studies, namely, van Buul-Offers, 12 mU; Holder, 13 mU; Schoenle, 14-28 mU; this paper, 21-46 mU. From these values it would appear that roughly the same doses of IGF activity produce a significant body weight gain in hypophysectomized rats as in Snell dwarf mice.

In view of the ability of unfractionated human plasma or serum (ref. 19) to produce a growth response in hypophysectomized rats, the question arises as to how much of this response is accountable by the content of IGF in plasma or serum. In tibial assays of pooled human plasma, a 40% increase in epiphyseal cartilage width was achieved in 4 days by the daily injection of 9 ml of plasma (0.2 mU IGF per ml) for 4 days, or a total of about 10 mU of IGF. In the present work, however, a daily dose of 21 mU of IGF for 8 days, or a total of 168 mU, was required to achieve the 40% increase. The 17-fold higher ratio of growth promoting activity in plasma relative to its IGF content suggests that IGF alone cannot account for the entire growth

promoting activity of plasma. This would indicate that other plasma factors such as the IGF carrier protein, platelet-dependent growth factors (ref. 20), and fibroblast growth factor (ref. 21) may be needed to augment or synergize the response to IGF.

An additional finding of interest is that IGF, in contrast to GH (refs. 2 and 3), does not induce formation of the large molecular weight IGF carrier protein in serum (fig. 4). The carrier protein binds most of the IGF present in serum (ref. 22) and is likely to be responsible for the long half-life of IGF (ref. 23). IGF injected intravenously as a bolus causes a profound hypoglycemia that is readily demonstrable in adrenalectomized rats (ref. 24). In the absence of the carrier protein, the slow continuous subcutaneous infusion by means of the Alzet pumps appears to have delivered enough IGF to the cells responsible for growth but not enough to stimulate the insulin target tissues. It is possible that the IGF-mediated aspects of growth hormone action cannot be completely mimicked with the free IGF peptide because the hypophysectomized animal lacks the large molecular weight binding protein.

In summary, it has been shown that IGF has significant growth promoting effects in pituitary deficient rats thereby providing direct evidence in favor of the somatomedin concept, as originally formulated by Daughaday et al. (ref. 1).

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TABLE 1.- ORGAN AND BODY WEIGHTS AT AUTOPSY

Treatment	Daily dose	Body weight		Liver, mg	Thymus, mg	Fat pads			Soleus, mg	Testes, mg
		g	Δg			Epididymal, mg	Retro- peritoneal, mg			
Control (5) ^a	0	91.4	0	3.83 ±0.20	258 ±24	280 ±33	193 ±48	--	166 ±6	
hGH (4)	86	113.8	17 ±0.9 ^b	5.27 ±0.31 ^b	386 ±47 ^c	245 ±41	188 ±32	--	191 ±21	
IGF (5)	21	97	4.2 ±1.6	4.37 ±0.10	332 ±25	234 ±23 ^c	156 ±30	--	198 ±23	
Control (3)	0	91	0	3.50 ±0.30	172 ±34	281 ±58	124 ±23	41 ±2.0	206 ±17	
IGF (3)	46	115	11.7 ±2.8 ^b	4.68 ±0.25 ^c	278 ±9 ^c	183 ±26	97 ±44	53 ±2.8 ^c	130 ^d	
Control (3)	0	103	8 ±1.0	4.38 ±0.25	268 ±13	--	--	45 ±3.2	168 ±9.5	
hGH (4)	48	121	23 ±2.2 ^b	5.33 ±0.43	507 ±57	255 ±26	--	56 ±3.0 ^e	194 ±15	
IGF (4)	6	100	2.3 ±1.0	4.27 ±0.5	299 ±42	183 ±30 ^c	--	43 ±3.2	150 ±16	

^aNumber of rats per group given in parentheses.^bp < 0.01.^cp < 0.05.^dFrom two rats.^ep < 0.1.

Values presented are means ± standard errors.

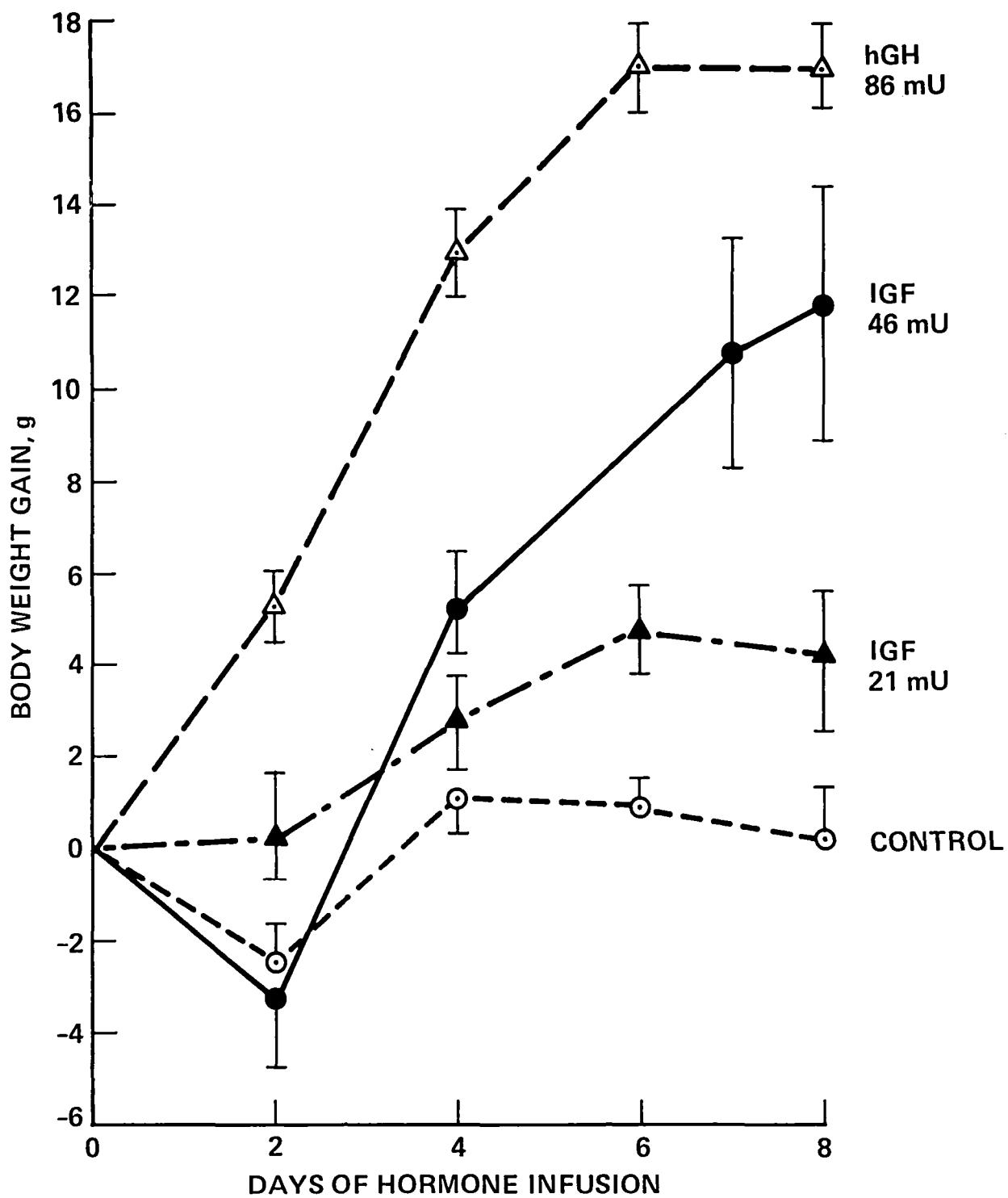


Figure 1.- Increases in the body weight of hypophysectomized rats infused for 8 days with IGF at the rate of either 21 or 46 mU/day and hGH at the rate of 86 mU; control rats were infused with an equal weight of human serum albumin. There were four or five rats per group except for highest dose IGF group which contained three rats.

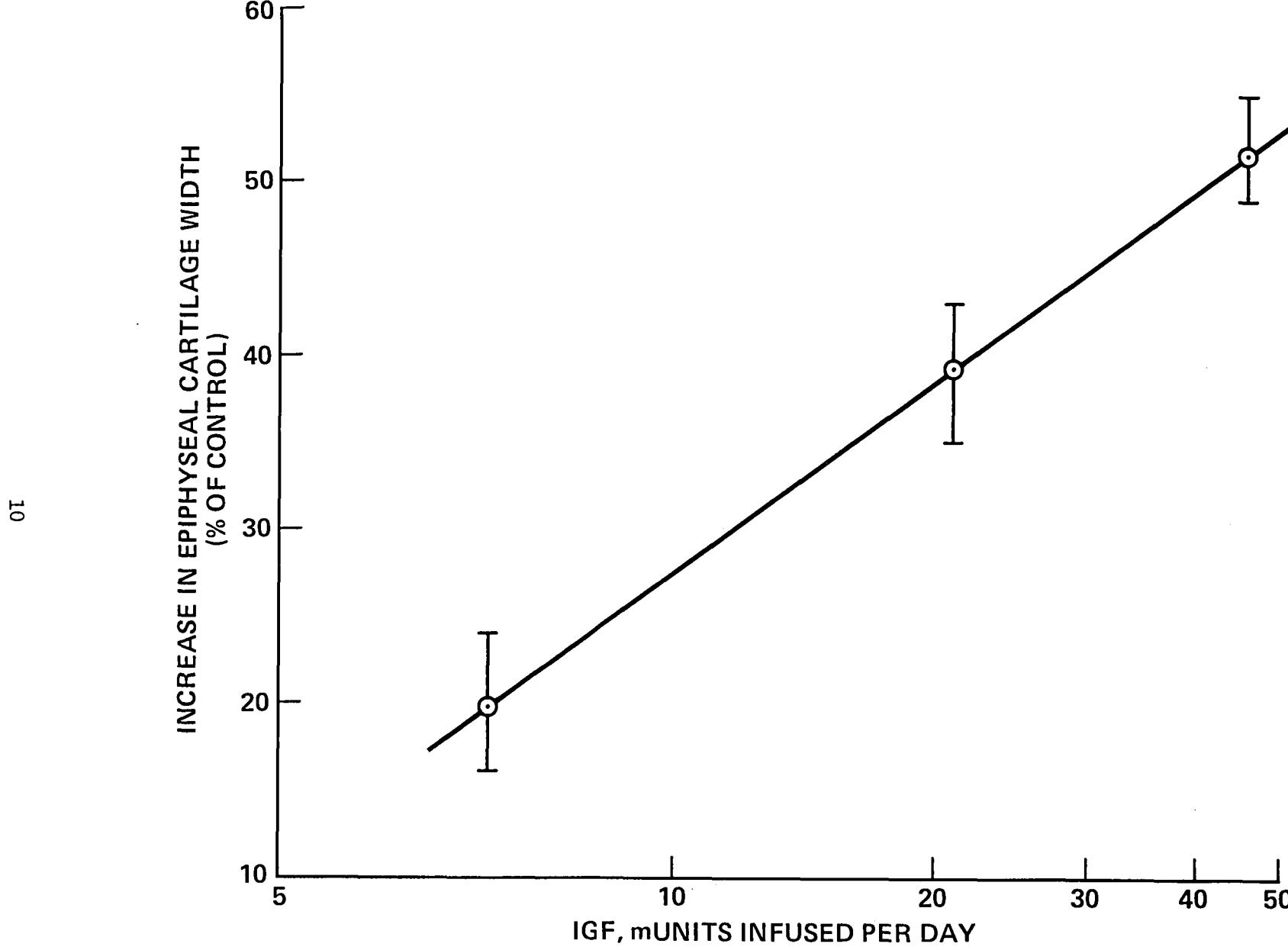
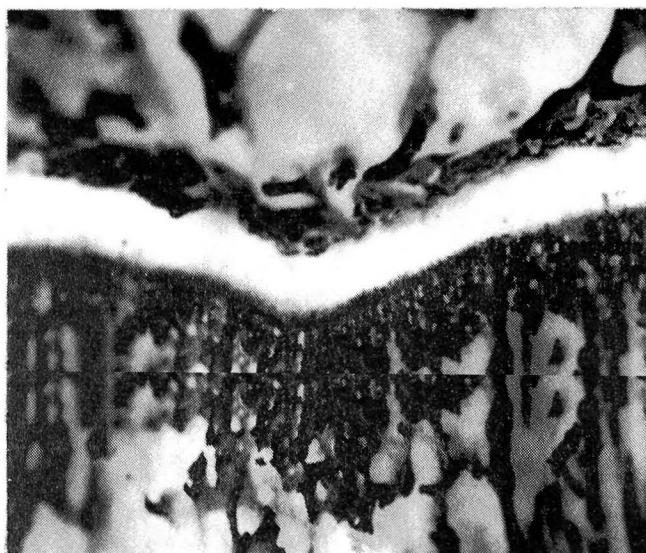


Figure 2.- Response of epiphyseal cartilage width to the log of the dose of IGF infused per day for 8 days. Response expressed as percentage increase of control rats infused with human serum albumin. The infusion of 86 mU of hGH increased the cartilage width by 100%.



Control



IGF-treated



hGH-treated

Figure 3.- Proximal epiphyseal cartilage from hypophysectomized rats showing stimulation of cartilage growth and calcification in zones of provisional calcification and primary spongiosa. Controls were infused with human serum albumin, IGF treated with 21 mU/day, and hGH treated with 86 mU/day; stained with 1% silver nitrate according to reference 9.

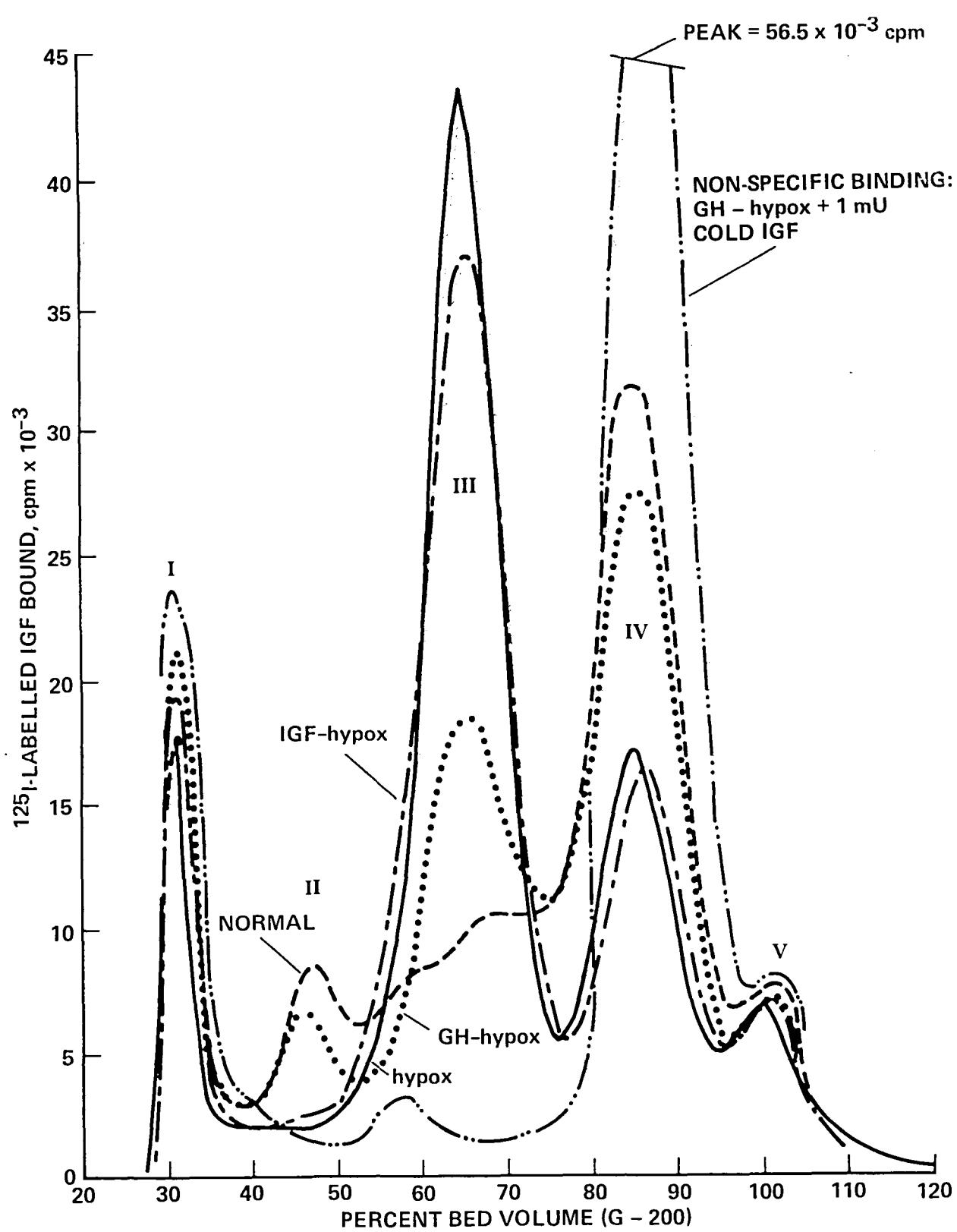


Figure 4.- Distribution of ^{125}I -labeled IGF I in effluent fractions obtained by gel permeation of rat sera on Sephadex G-200 at pH 7.4; ^{125}I -IGF I (7.8×10^6 cpm) was equilibrated for 24 hr at 4°C in 0.25 ml of Dulbecco's buffer, pH 7.4 and 0.25 ml of pooled sera (three rats) from the following groups of hypophysectomized rats: IGF-hypox, infused with 46 mU of IGF per day for 8 days; GH-hypox infused with 86 mU of hGH for 8 days; hypox, infused with saline containing human serum albumin equivalent to the weight of IGF, for 8 days. The mixture was then applied to a Sephadex G-200 column (2×60 cm, bed volume 190 ml) equilibrated with Dulbecco's buffer, pH 7.4; 2.2 ml fractions were collected and counted in a liquid scintillation counter.

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